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Natural and anthropogenic forcing of Holocene lake ecosystem development at Lake Uddelermeer (The Netherlands)

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Abstract

Lake Uddelermeer (The Netherlands) is characterized by turbid conditions and annual blooms of toxic cyanobacteria, which are supposed to be the result of increased agricultural activity in the 20th century AD. We applied a combination of classic palaeoecological proxies and novel geochemical proxies to the Holocene sediment record of Lake Uddelermeer (The Netherlands) in order to reconstruct the natural variability of the lake ecosystem and to identify the drivers of the change to the turbid conditions that currently characterize this lake. We show that the lake ecosystem was characterized by a mix of aquatic macrophytes and abundant phytoplankton between 11,500 and 6000 cal yr BP. A transition to a lake ecosystem with clear-water conditions and relatively high abundances of 'isoetids' coincides with the first signs of human impact on the landscape around Lake Uddelermeer during the Early Neolithic (ca. 6000 cal yr BP). An abrupt and dramatic ecosystem shift can be seen at ca. 1030 cal yr BP when increases in the abundance of algal microfossils and concentrations of sedimentary pigments indicate a transition to a turbid phytoplankton-dominated state. Finally, a strong increase in concentrations of plant and faecal biomarkers is observed around 1950 AD. Canonical Correspondence Analysis (CCA) suggests that reconstructed lake ecosystem changes are best explained by environmental drivers that show long-term gradual changes (sediment age, water depth). These combined results document the long-term anthropogenic impact on the ecosystem of Lake Uddelermeer and provide evidence for pre-Industrial Era signs of eutrophication.

Introduction

The majority of water bodies in the world has to some degree been affected by human impact, and pollution of freshwater ecosystems is considered a global problem (Carpenter et al. 1998; Smith et al. 1999; Smol 2008). It is generally assumed that lake ecosystems in northwest Europe underwent a phase-shift from clear-water to a turbid state dominated by phytoplankton as a result of increased nutrient input in the mid-20th century, which by itself was a consequence of intensified agriculture (Carpenter et al. 1999; Van der Molen and Portielje 1999; Scheffer et al. 2001; Nienhuis et al. 2002). Indeed, many studies show evidence for recent eutrophication of lake ecosystems as a result of modern agricultural practices and urban development (Rasmussen and Anderson 2005; Kirilova et al. 2010a; Millet et al. 2010; Wiik et al. 2015). However, the population increase, forest disturbance, and settlement of the first farming communities during the Mesolithic to Neolithic transition (6400-6000 cal yr BP; Woodbridge et al. 2014) might have already enhanced nutrient loading of lakes (Bradshaw et al. 2005a). Several case studies have shown human-driven changes on the trophic status of lakes from 4000 cal yr BP onward (Dapples et al. 2002; Lotter and Birks 2003; Bradshaw et al. 2005b; Hillbrand et al. 2014).

Lake Uddelermeer (The Netherlands; Fig. 1) is described in historical documents as a clear-water 'idyllic' site (van Eeden 1886), but over the last decades it has had annual blooms of toxic cyanobacteria (*Microcystis* spp.). Similar to many other Dutch lakes, Lake Uddelermeer is now a turbid, nutrient-rich site with only few macrophytes, with total phosphorus levels exceeding 100 µg L⁻¹ and with Chlorophyll *a* levels over 25 µg L⁻¹ in summer (Grontmij 1996). It has been suggested that eutrophication of the lake is the result of manure-derived nutrient input into the lake during the second half of the 20th century (Grontmij 1996). Palynological results by Bohncke (1999) indicated that there were changes in the late Holocene aquatic vegetation of the lake, but Bohncke (1999) was not able to establish when or why cyanobacteria had become abundant constituents of the lake ecosystem.

Over the last two decades there has been major progress in laboratory analytical techniques and their application in palaeoecological research. It is now possible to quantify trace concentrations (µg) of different types of molecules that indicate their source (e.g. geochemical biomarkers) using chromatographic approaches. The development of sedimentary pigment analysis using high performance liquid chromatography (HPLC) now enables us to establish changes in the phytoplankton community that cannot be reconstructed using microfossil analysis (McGowan 2013). Furthermore, the possibility to directly establish input of animal wastes into a lake through analysis

of faecal biomarkers (i.e. 5 β -stanols; Bull et al. 2002) provides for a direct means to test whether changes in a lake ecosystem were triggered by changes in manure input.

The aim of this paper is to investigate long-term (Holocene) ecological developments in Lake Uddelermeer as a response to natural and anthropogenic changes. We hypothesize that intensification of agricultural practices in the mid-20th century has resulted in a major ecological shift in the lake, changing from an oligotrophic clear-water lake to a eutrophied turbid lake dominated by abundant phytoplankton. We will combine palaeoecological analytical techniques with sedimentary pigment analysis to establish when the lake underwent ecological changes, and will statistically compare our results to changes in several environmental factors, including the increase in manure input that will be established using plant and faecal biomarker analysis.

Study site

Lake Uddelermeer (52°14'48"N, 5°45'40"E; Fig. 1) is a classic study site for Dutch palaeoecologists, as it is the only site in the Netherlands to have continuously registered climatic and environmental change from the late-glacial up to the present. The relatively thick sediment infill of lake Uddelermeer of ~15.6 m allows for detailed analyses of past environmental change. The lake's sediment record was first studied by Polak (1959), and has been re-cored a few times since Polak's earliest work, including a field-campaign in the middle of the 1980s (Bohncke et al. 1988), the late 1990s (Bohncke 1999), and most recently in 2012 in the context of a study of Late Holocene lake level fluctuations (Engels et al. 2016).

The lake forms a focal point for regional groundwater flow as it is situated in a relatively low position (24 m asl) in the landscape between two ice-pushed ridges of Saalian age, which reach a maximum height of over 100 m asl in the surroundings of Lake Uddelermeer. A pingo formed at the location of Lake Uddelermeer during the Last Glacial Maximum and subsequently disappeared as a result of climate warming. A lake formed in the depression that was left in the landscape, and sediment accumulated in the lake from ca. 14,000 cal yr BP onward (Engels et al. 2016). The lake currently measures approximately 200 by 300 m and has a maximum water depth of 1.3 m.

The direct surroundings of Lake Uddelermeer are managed by the 'Royal Domain Het Loo', which strives for a natural type of forestry. The management-style has resulted in the presence of a park-landscape with oak, beech and pine on the elevated sandy soils to the north and east of the lake. The valley in which Lake Uddelermeer is situated is characterized by intensive agriculture, whereas the ice-pushed ridges themselves are mostly covered by coniferous forests, planted in areas that were previously characterized by open heathland. The lake itself is currently devoid of

submerged macrophytes and only a few floating-leaved vegetation stands (e.g. *Nymphaea alba* L.) are present in the lake. The lake is bordered by stands of trees (mostly *Alnus*) on the west and a small fringe of wetlands to the north, northeast, and south, with willow and birch as the main arboreal taxa. Extensive emergent stands of *Phragmites australis* (Cav.) Trin. ex Steud. are present along the eastern edge of the lake (Engels et al. 2016; van den Bos et al. 2017).

Material and methods

Sediment record and age-depth modelling

A series of sediment cores was retrieved from the lake during fieldwork in April and May 2012. Here we present results obtained from core sequence UDD-E, which was extruded from the deepest part of the sedimentary basin using a 3-m-long UWITEC piston corer deployed from a floating platform. Although core UDD-E reached down to 1500 cm sediment depth, we here only focus on the Holocene part of the sediment sequence (i.e. 0-1300 cm). We retrieved the uppermost part of the sediment sequence using a Niederreiter gravity corer (0-67 cm) and combined the gravity core with the long piston cores by comparing their loss-on-ignition profiles, which were obtained using consecutive 1 cm thick samples (Engels et al. 2016). An age/depth-model (Fig. 2) was constructed for this core sequence by Engels et al. (2016) based on radiocarbon dating ($n = 20$) and ^{210}Pb dating (for the upper part of the record) and using Bayesian modelling as included in OxCAL (Bronk Ramsey 2009). Ages are reported here in calibrated years before present (BP), where 'present' refers to 1950 AD, and rounded to the nearest decade. In this paper we will use the stratigraphical classification of the Holocene as suggested by Walker et al. (2012) and the archaeological periods as defined for the Netherlands (www.cultureelerfgoed.nl).

Elemental analysis

A total number of 92 samples was taken from the sediment core for elemental analysis, using a 20-cm sampling interval for the lower part of the sequence (1295-505 cm) and a 10-cm interval for the upper part of the sediment (495-0 cm), with the addition of two extra samples in the top-part of the sequence (0.5 and 1.5 cm depth) to cover the most recent history of the lake.. For each sample, 5 to 10 mg of freeze-dried, homogenized material was weighed into a tin foil capsule and analysed. We used an Elementar VarioEL elemental analyzer to obtain data on total carbon and nitrogen content and performed each analysis in duplicate. Sample combustion took place at 1150 °C and complete

oxidation was realized by supplying 99.99% oxygen to the combustion process for 90 sec. After combustion the released CO₂, H₂O, NO_x and SO₂ gases were carried by 99.99% helium to a thermal conductivity detector (TCD). Before detection the NO_x gases were reduced to N₂ by passing through a copper column at 850 °C. The analyses were calibrated using sulfanilic acid (C₆H₇NO₃S). The sulphur data were omitted from this study because the sulphur column was not functioning. As loss-on-ignition on 950 °C indicates there is no inorganic carbon present in the sediment, we assume that the total carbon as measured by the elemental analyzer is equivalent to total organic carbon (OC).

Sedimentary pigment analysis

Sedimentary pigment analyses were performed on samples obtained from the same depths as those used for the elemental analyses. After freeze-drying and homogenization, ~100 mg of sediment was disrupted for 60 seconds in a BioSpec Mini BeadBeater at maximum speed using ~0.5 g of 0.5 mm silica beads and 1000 µl of 80:15:5 acetone:methanol:ultrapure water (v/v/v), after which the samples were incubated for 12 hours at 4°C. Subsequently, 500 µl ion-pair mix consisting of 1.5% tributyl ammonium acetate and 7.7% ammonium acetate in ultrapure water (MilliQ) was added to the sample. After centrifuging (15,000 rpm, 3 min) the supernatant was stored at -20 °C until HPLC analysis.

All pigment analyses were conducted on a Shimadzu HPLC using a Thermo Scientific ODS Hypersil column (250 x 3 mm, particle size 5 µm) and a KONTRON SPD-M20A diode array detector using a linear gradient from 100% elution mix A (methanol:acetonitril:1 M ammonium acetate in MilliQ (50:25:25 v/v/v)) to 100% elution mix B (acetonitril:acetone:methanol (60:20:20 v/v/v)).

Standards of 15 different pigments were used for identification of unknown pigments using retention time and absorption spectrum. The concentrations were corrected for the total organic carbon content of the sediment as determined using the elemental analyzer. The pigments zeaxanthin and lutein are isomers with an equal retention time and are therefore difficult to separate. However, since they have different absorption spectra they were separated on the basis of the wavelength shift of the left peak in the absorption spectrum, using calibration mixes containing 100:0, 75:25, 50:50, 25:75 and 0:100 (w/w) of resp. zeaxanthin and lutein standards. The pigment data were plotted using C2 version 1.7.4 (Juggins 2011) and a Principal Component Analysis (PCA) with standardized and centered variables was used to produce a summary-curve of the sedimentary pigment data.

Sterol and stanol analysis

Samples were taken for sterol and stanol analysis at 1-m intervals in the lower part of the core (1295-405 cm), and at 20-cm intervals in the upper part of the core (395-0 cm), with the addition of two extra samples in the top-part of the sequence. This resulted in a total of 30 samples with a relatively high sampling resolution in the late Holocene part of the record but with fewer samples in the older interval. Sterols and stanols were analyzed using a modified version of the method by Evershed et al. (1997). An average of 0.720 g of freeze-dried sediment was homogenized and analyzed in duplicate from the extraction (if enough material was available).

Accelerated solvent extraction (ASE) using dichloromethane (DCM):methanol (97:3 v/v) was used to extract the total lipid extract (TLE) from the sediment samples under 75 °C and 2500 kPa for 5 min. The TLE was desulfurized and separated into acid and neutral fractions using bonded aminopropyl solid phase extraction cartridges. The neutral fraction was fractionated into 4 lipid fractions that were eluted from activated silica columns with increasingly polar solvents: esters (F1); ketones (F2); alcohols (F3); and fatty acids (F4). The alcohol fraction (containing the sterol compounds) was subsequently silylated using 100 µl BSTFA/TMCS at 70 °C for 60 min, evaporated to dryness under N₂, and redissolved in 400 µl of cyclohexane. A subsample of 200 µl was used for full scan gas chromatography-mass spectrometry (GC-MS) analysis, and a further 200 µl of each sample was used for selective ion monitoring (SIM) GC-MS analysis using m/z 215 to identify 5β-stanols.

All GC-MS analyses were carried out on a ThermoQuest Trace GC 2000 gas chromatograph connected to a Finnigan Trace quadrupole mass spectrometer. The system was cooled secondarily for 3 minutes prior to injection. The temperature program after injection consisted of an initial temperature of 50 °C for 2 min, heating at 60 °C min⁻¹ to 80 °C, holding at 80 °C for 2 min, heating at 20 °C min⁻¹ to 130 °C immediately followed by heating at 4 °C min⁻¹ to 350 °C and finally holding at 350 °C for 10 min.

The sterol and stanol compounds were identified using their mass spectra and retention times. The analytes were quantified using the response factor of external standards that were acquired from Sigma-Aldrich, and analyte quantities were corrected for the total organic carbon content of the sediment as determined using the elemental analyzer. A diagram showing concentrations of individual sterols and stanols was plotted using C2 version 1.7.4 (Juggins 2011).

The trivial names of the analytes are used in this paper. We use the following information for the interpretation of the sterol and stanol compounds: sitosterol, stigmasterol and campesterol are the most abundant plant sterols, and cholesterol is the most abundant sterol in animals, but its concentrations are negligible in plants. Cholesterol and its biohydrogenation products coprostanol and epicoprostanol are therefore likely indicators of animals with a mixed diet containing high levels

of cholesterol (e.g. humans, pigs). The most abundant steroid lipids in herbivore animal dung are 5 β -stigmastanol, and its 3 α -epimer, and 5 β -epistigmastanol, which are the products of anaerobic microbial transformation of sitosterol by biohydrogenation during herbivore digestion (Eyssen et al. 1973; Evershed et al. 1997; Bull et al. 2002). Similar microbial hydrogenation products are produced for campesterol (5 β -campestanol and 5 β -epicampestanol) and stigmasterol (24-ethyl-cholest-22-en-3 β -ol and 3 α -epimer). A wide range of anaerobic and facultative anaerobic microbial species are capable of transforming, and have a requirement for, Δ 5 sterols (Eyssen et al. 1973; MacDonald et al. 1983). 5 α -stigmastanol indicates that a significant aerobic microbial reduction of sitosterol (Bull et al. 2002) has occurred after stigmasterol is deposited in the environment. They may therefore be used as biomarkers in palaeoecological studies using lacustrine sediments (Cheng et al. 2016).

Palaeoecological analysis

Palynology

Seventy-eight volumetric samples (0.7 cc) were selected from the Holocene sediment of Lake Uddelermeer and prepared for palynological analyses using standard techniques (Faegri and Iversen 1989; Moore et al. 1991). A reference collection as well as keys and illustrations by Moore et al. (1991) and Beug (2004) were used for pollen identification. The identification of fungal spores and other non-pollen palynomorphs (NPPs) follows Van Geel (1978). Engels et al. (2016) provide a description of the (late-glacial as well as Holocene) pollen record for Lake Uddelermeer, and interpret the observed changes in the context of regional vegetation development and climate change. In this manuscript we re-analysed the data by Engels et al. (2016) in the context of lake ecosystem development. We describe within-lake conditions by particularly focusing on changes in the records of aquatic macrophytes as well as on changes in NPPs derived from aquatic algae (van Geel 2001). Additionally, we provide and interpret records of terrestrial taxa that might be indicative of erosion, farming (including the retting of hemp, traceable in the palynological record of Lake Uddelermeer by the record of *Cannabis* pollen; Engels et al. 2016) or other processes that might have impacted the lake ecosystem. The 'Cannabis type' presented in this study includes pollen of *Cannabis sativa* L. as well as of *Humulus lupulus* L. However, close examination of a number of *Cannabis*/*Humulus*-type grains using the criteria by Punt and Clarke (1984) indicates that they most likely derived from *C. sativa*, and their sudden appearance in high abundances in the upper part of the record suggests that these pollen grains were most likely deposited as the result of retting of

1 hemp in the lake (Slicher van Bath 1987; Engels et al. 2016). All encountered microfossils are
2 expressed as a percentage of the pollen sum, which includes pollen of trees and shrubs, Ericaceae
3 and upland herbs.
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6 *Chironomid analysis*

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10 In addition to the palynological dataset, we here re-interpret the chironomid record presented by
11 Engels et al. (2016). As chironomid analysis is more time-demanding and, in the case of our record,
12 needed higher volumes of sediment than e.g. the palynological analysis, a subset of 32 samples was
13 selected for chironomid analysis, covering the upper half of the record only. Samples were treated
14 with warm KOH for 15-30 minutes and subsequently rinsed over 100 µm meshes. Chironomid head
15 capsules were hand-selected from the residues using a sorting tray and a stereomicroscope (35x
16 magnification). After air-drying the head capsules were mounted on permanent chironomid slides
17 using Euparal mounting medium and identified using Brooks et al. (2007). A chironomid percentage-
18 abundance diagram was constructed and a PCA was run on the percentage data to summarize the
19 main trends in the data using C2 software (Juggins 2011). In this paper we focus on ecological traits
20 of some of the key taxa that were encountered in the record, with a special emphasis on habitat
21 preferences as observed in modern data in the Netherlands and beyond (Moller Pillot and
22 Vallenduuk 2007; Moller Pillot 2009: 2013)
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34 Numerical analysis

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39 Redundancy analysis (RDA) and Canonical Correspondence Analysis (CCA) have been used in
40 palaeoecology to statistically model and evaluate the effects of environmental forcings on
41 paleoecological datasets (Lotter and Birks 1993). Using Monte Carlo permutation tests, these
42 techniques can assess whether shifts in stratigraphical records associated with 'events' are
43 statistically significantly different from random variations. Additionally, the influence of co-variables
44 can be 'partialled out' using RDA/ CCA. Examples of the application of these techniques to
45 palaeoecological datasets include tests of the statistical significance of the impact of a volcanic
46 eruption on the regional vegetation and on the diatom flora of lakes (Lotter and Birks 1993; Birks
47 and Lotter 1994; Engels et al. 2015) and the identification of drivers of long-term pollution trends in
48 lakes (Bradshaw et al. 2005b).
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56 We combined the fossil pigment dataset and the percent-abundance curves of the aquatic
57 taxa (aquatic macrophytes and algae) to a response variable dataset here termed the 'aquatic
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dataset', using standardized and centered data. Not all datasets included the same number of samples, so we only used those samples that were analyzed for fossil pigments, pollen and plant and faecal biomarkers. For the one depth-interval where a sample was taken for sterol and stanol analysis, but no sample was prepared for palynological analysis (55 cm depth: 37 cal yr BP), we performed a linear interpolation between the adjacent pollen samples to produce pollen data that matched the sterol and stanol sampling scheme. A detrended correspondence analysis (DCA) using Hellinger data-transformation was applied to this dataset of 'response variables' and showed the gradient length along axis 1 to be 2.00 standard deviations. We therefore chose an ordination technique that assumes unimodal models (CCA) in the subsequent analyses (Birks 1998).

We used the following variables as our explanatory dataset: (1) age, representing long-term developments and forcing mechanisms (e.g. insolation changes) following Birks and Lotter (1994); (2) total percent-abundance of tree pollen (Arboreal Pollen; AP), representing regional vegetation and land-use change; (3) organic carbon content of the sediment, representing catchment-related processes; (4) percent-abundance of *Cannabis* pollen, representing the effects of retting of hemp; (5) the scores on the first axis of a PCA ran with the standardized and centered sterol and stanol concentration data, representing the influx of faecal matter; and (6) past water depths as reconstructed using the lake-level record by Engels et al. (2016) and the sediment depth, assuming no changes in compaction rates through time. We used a series of (partial) CCAs to assess which ecological driver(s) had a statistically significant impact on the aquatic ecosystem of Lake Uddelermeer. All ordination analyses were performed using the vegan library in R (Oksanen et al. 2016) using analysis of variance (ANOVA) with 999 permutations to assess significance.

CCA of the response dataset with age as an explanatory variable showed that this variable explains a significantly part of the variation in the response data. Therefore, in subsequent ordinations we used age as a co-variable in order to partial out the variation related to age and so make the variation explained by other factors easier to interpret. The explanatory variable water depth shows strong co-variation with age (as determined using the cor.test function in R) as lake-infilling progressed with time, and also explained a significant part of the variance of the response data when used as an explanatory variable in CCA. We ran a third set of analyses using water depth as a co-variable. As the results are similar to those obtained using age as a co-variable, we only present the results of the runs with age as an explanatory variable here. Finally, no tests combining the effects of AP and *Cannabis* percentage were carried out, as these two explanatory variables are both used in the calculation of the pollen sum against which they are expressed, and are therefore not independent.

As our results indicated that the largest ecosystem shifts occurred in the upper part of the record, we created a truncated dataset which covered the uppermost 20 samples, spanning the time-interval between 1710 cal yr BP and the present. Following Bradshaw et al. (2005b) we explored if drivers of recent ecosystems change differed from Holocene-scale drivers of change by re-running all analyses using the truncated dataset.

Zonation of our diagrams was carried out using a constrained sum-of-squares cluster analysis as implemented in the rioja package in R (Juggins 2017). We used the square-root transformed aquatic dataset in order to identify major changes in assemblage composition, and assessed statistical significance of the indicated zonal transitions by broken stick modelling (Bennett 1996). The results indicate the occurrence of one statistically significant zonal boundary at 1030 cal yr BP. We visually established another transition in our aquatic dataset at 6000 cal yr BP, when indicator taxa such as *Isoetes* for the first time appear in the record, albeit in low percent-abundances.

Results

Chronology

The age-depth model for the central core sequence from Lake Uddelermeer (Fig. 2) shows poorly constrained age-estimates for the older part of the record (ca. 13,700 to 4500 cal yr BP) due to the limited amount of material suitable for radiocarbon dating in this part of the record (approximately 1540-800 cm core depth). The chronology for the interval 4500-3000 cal yr BP shows error estimates of ± 500 years, and error estimates of ± 200 years from 3000 cal kyr to the present. The age-depth model of the upper part of the record is considered reliable as long as these error estimates are taken into account. Relatively low accumulation rates of approximately 0.9 mm yr^{-1} can be deduced from Fig. 2 for the period between 13,700-2800 cal yr BP, with increased accumulation rates of approximately 2.1 mm yr^{-1} for the period between 2800 cal yr BP and the present.

Sedimentary pigments

All analyzed pigments show relatively low concentrations during the oldest part of the record (11,700-10,200 cal yr BP), with the exception of chlorophyll *a* and *b* (Fig. 3). Pigments can degrade as a result of exposure to oxygen, light and heat (Leavitt 1993), where chlorophylls tend to be relatively labile, whereas in general carotenoids are more stable (McGowan 2013). The relatively high

abundance of chlorophylls in the oldest part of the record suggests that pigment degradation at Lake Uddelermeer was relatively limited. The majority of pigments degrade in the water column due to e.g. microbial oxidation, digestion by invertebrates or bacterial degradation, and pigment degradation can continue in oxygenated sediments. As Lake Uddelermeer has had relatively high accumulation rates (1-2 mm yr⁻¹), we hypothesise that the pigments were buried beneath the zone of bioturbation and oxygenation relatively quickly when compared to other lakes. Alloxanthin contents increase from 7.7 µg g⁻¹ OC at 10,560 cal yr BP to 31 µg g⁻¹ OC at 10,200 cal yr BP, reaching peak values of 72 µg g⁻¹ OC at 9900 cal yr BP, after which it shows a decreasing trend into the mid-Holocene. A similar trend can be observed for zeaxanthin, which shows peak values of 142 µg g⁻¹ OC at 9600 cal yr BP. Diatoxanthin also shows an increase in the early Holocene, but unlike alloxanthin and zeaxanthin it does not show a decreasing trend in the early to mid-Holocene. Instead, diatoxanthin shows relatively high contents per total organic carbon up to 6400 cal yr BP. All individual pigments show relatively low values in the interval 6400-1120 cal yr BP, at which point most pigments show an abrupt increase in content. For instance, chlorophyll *a* increases from <1 µg g⁻¹ OC to values over 10 µg g⁻¹ OC at 1120 cal yr BP. Alloxanthin and chlorophyll *b* are the only two pigments that do not show an abrupt increase at 1120 cal yr BP, where the latter only shows peak contents of 4-9 µg g⁻¹ OC in the uppermost four samples of the sequence (-50 cal yr BP (2000 AD) to the present).

Chlorophyll *a* and *b* show relatively high values in the uppermost samples of the sequence. However, the interpretation of fossil pigment signals in such young sediments is problematic as it is not possible to distinguish elevated concentrations of pigments deriving from increased algal abundance, from pigments that are still present due to incomplete *in situ* degradation (Leavitt 1993; Hall et al. 1999). In addition, the uppermost eight pigment samples were retrieved using gravity coring, and have been exposed to light longer than the material that was obtained using a piston corer, potentially leading to additional degradation of the pigments in these samples.

Plant and faecal biomarkers

The records of the individual sterol and stanol compounds show an initial increase of relatively low concentrations in the oldest sample(s) of our record to slightly increased levels during the period 10,000-8000 cal yr BP. This includes increases in 5 α -campestanol (from approximately 13 to 28 µg g⁻¹ OC), 24-ethylcoprostanol (from 2-8 to 24 µg g⁻¹ OC) which are clearly visible in Fig. 4, but also in coprostanol (from 0.5 to 0.8 µg g⁻¹ OC) and epicoprostanol (from 2.0 to 4.3 µg g⁻¹ OC.) Most of the sterol and stanol records subsequently show a return to relatively low values, which remain stable to

1 ca. 1270 cal yr BP. 24-ethyl-5B-cholestan-3 α -ol does show variable concentrations throughout the
2 early and mid Holocene, and the increased concentrations of Epicoprostanol at 2140 cal yr BP, likely
3 indicates low inputs of plant and faecal matter to the lake prior to ca. 1270 cal yr BP. While it is likely
4 that the relatively high accumulation rates across the Holocene led to favorable depositional
5 conditions, it is possible that degradation processes were more intense during some parts of the
6 Holocene. More intense degradation during the early to middle Holocene, when summer
7 temperatures were higher than today, could have led to the observed low values for many of the
8 sterol and stanol records.

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10 Increases in herbivore faecal biomarker concentrations can further be seen at ca. 1270 cal yr
11 BP, when 5 β -campestanol increases from 11.2 to 16.5 $\mu\text{g g}^{-1}$ OC. This increase is followed by a
12 second increase of 5 β -campestanol at ca. 1100 cal yr BP. Stigmasterol increases from 24 $\mu\text{g g}^{-1}$ OC at
13 ca. 1200 cal yr BP to values over 300 $\mu\text{g g}^{-1}$ OC after 940 cal yr BP and is interpreted as an indicator of
14 input of plant matter to the lake (Volkman 1986). Epicoprostanol, cholestanol and β -sitosterol all
15 show a strong increase in concentrations across the interval 1100-940 cal yr BP too.

16
17 The most striking feature of the record is the strong increase in concentrations that is seen
18 in almost all analytes in the uppermost two samples of the record, dating to -50 and -62 cal yr BP
19 respectively (i.e. 2000 and 2012 AD). For instance, coprostanol increases from values below 1 $\mu\text{g g}^{-1}$
20 OC to values of 14-19 $\mu\text{g g}^{-1}$ OC. The recent formation of these sediments might mean that
21 degradation processes did not yet have the same effects as they had on older sediments located
22 further down the core, potentially partly explaining the very high concentrations observed in the
23 upper part of the record (2000 AD – present).

24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 Palaeoecological results

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42 The main pollen diagram (Fig. 5) shows an initial Holocene phase with very high arboreal pollen
43 percentages (over 90%) and only small contributions of non-arboreal pollen (NAP). This phase most
44 likely reflects the Mesolithic (ca. 11.7-7.2 cal kyr BP) and early Neolithic (ca. 7.2-6.3 cal kyr BP)
45 periods where the landscape was forested and human impact was relatively low. A decrease in the
46 AP/NAP-ratio from 6000 cal yr BP onward likely reflects the onset of stronger human impact on the
47 landscape, probably through forest cutting for agricultural purposes and a lack of regeneration due
48 to domestic grazing. The first occurrences of crop plants (Cerealia) are registered from 6000 cal yr BP
49 onward too. The AP/NAP-ratio shows a decreasing trend resulting in AP-values of ~50% during the
50 Roman Period (12 BC – 450 AD), after which a short phase of forest recovery is registered during the
51 early Medieval period (450-800 AD). Rapid deforestation is seen from 1500 cal yr BP onward, where
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pollen of crop plants (*Cerealia*, *Secale*) increase in abundance. A strong increase in *Cannabis* pollen can be seen at ~450 cal yr BP, which most likely indicates a period of retting of hemp in the lake (Slicher van Bath 1987; Engels et al. 2016).

The aquatic vegetation shows several phases of development. Only few pollen and microfossils of aquatic macrophytes and algae are present in the lowermost part of our record (11,700- 6000 cal yr BP). An increase in *Littorella* and *Isoetes* is observed at 6000 cal yr BP, simultaneous with the decrease in AP/NAP-ratio (Fig. 5). The biggest change in the aquatic vegetation can be seen between 1120 and 940 cal yr BP, when several algal taxa show abrupt increases in their abundance. Around the same time, *Myriophyllum alterniflorum* DC. becomes the most abundant aquatic macrophyte in our microfossil record and *Isoetes* decreases in abundance.

The chironomid record shows two phases of distinct changes in the invertebrate fauna. First, an assemblage that is characterized by *Cladotanytarsus mancus*-type, *Lauterborniella* and *Endochironomus albipennis*-type is replaced by a fauna that has high abundances of *Parakiefferiella bathophila*-type and *Lauterborniella* around 3100 cal yr BP. Most taxa that show high abundances in the lower part of the chironomid record decrease in abundance at around 1000 cal yr BP, where *Procladius*, *Microtendipes pedellus*-type and *Ablabesmyia* appear or increase in abundance. Scores of samples of a PCA that was run with all encountered chironomid taxa shows relatively gradual changes across the entire time interval covered by the chironomid data, although some taxa show more abrupt changes in their individual curves (Fig. 5).

Numerical analysis

Table I shows the results of the (partial) CCAs that were used to evaluate the effects of different environmental forcings on the palaeoecological 'aquatic dataset'. The aquatic dataset is a combination of pollen and other microfossils derived from aquatic taxa, including algae, and the concentrations of sedimentary pigments, and it is interpreted to represent the Holocene developments of the aquatic ecosystem of Lake Uddelermeer. The results of the runs where a single explanatory variable was used to explain the variance in the full aquatic dataset (listed under (1) in Table I) indicate that both age and water depth explain a significant portion of the variation in the aquatic dataset. The runs under (2) show that when the long-term effects of age are 'partialled out', none of the individual predictor variables explains a significant proportion of variance of the aquatic dataset. Only when multiple variables are combined (run 3), using the AP-percentage, carbon content and the PCA-scores of the plant and faecal biomarker data, are we able to explain a significant proportion of the variance in the aquatic dataset when explanatory variable 'Age' is

1 partialled out. The results of the tests that were run using a truncated version of the aquatic dataset,
2 only using the samples that span the time-interval from 1710 cal yr BP to the present (n=20 samples)
3 show similar results with both age and water depth explaining a significant portion of the variance in
4 the dataset, and a combination of multiple environmental factors needed to explain a significant
5 portion of variance with 'Age' is partialled out (Table 1).
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10 Discussion

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14 Holocene development of the freshwater ecosystem at Lake Uddelermeer

15 16 17 18 *Zone 1a: 11,700-6000 cal yr BP*

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21 The onset of the Holocene in NW Europe was characterized by strong environmental change.
22 Chironomid-based reconstructions of July air temperatures indicate an increase of 2-3°C from the
23 Younger Dryas to the Preboreal in the Netherlands (Heiri et al. 2007), and model simulations as well
24 as proxy data indicate that temperatures remained at high levels until 6000 cal yr BP (Renssen et al.
25 2009). The vegetation in The Netherlands responded to the warming at the onset of the Holocene by
26 showing a succession of tree species (from light-demanding to predominantly shade-tolerant) during
27 the early to middle Holocene. Long-term reconstructions of natural forest ecosystems showed that
28 post-glacial forest biomass tended to build up and peaked after a phase of a few millennia, which
29 was subsequently followed by a phase of slow decreases in forest biomass and nutrient availability
30 (Wardle et al. 2004; Kuneš et al. 2011). It is possible that relatively high temperatures, combined
31 with the dynamic vegetation development, led to an initial increased input of nutrients into Lake
32 Uddelermeer followed by a subsequent decline. This would explain the succession of different
33 dominant phytoplankton taxa with initial high abundances of alloxanthin (Fig. 3), possibly indicating
34 high abundances of cryptophytes right after the onset of the Holocene (McGowan et al. 2005),
35 followed by a cyanobacteria-dominated community between 10-9 cal kyr BP as indicated by high
36 values of zeaxanthin (Leavitt 1993; Hall et al. 1999). This phase might represent the period with the
37 highest natural nutrient loading of the lake. The sedimentary pigment record suggests a diatom-
38 dominated phytoplankton population between ca. 9000 and 6000 cal yr BP (Fig. 3).
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53 The pollen record shows relatively low abundances of macrophyte pollen between 11.5-6 cal
54 kyr BP, with *Potamogeton* and *Nymphaea* as the most common aquatic macrophytes (0-5%), and
55 with a wetland border dominated by *Typha angustifolia*/ *Sparganium*-type (Fig. 5). The relatively low
56 abundance of pollen of aquatic macrophytes might be a reflection of the high water depth (and
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hence low light availability) of the lake at this time, which may have limited colonization by submerged plants (typically occurring in depth ranges <2m and up to 6-10m; Rasmussen and Anderson 2005). As macrofossil analysis is better able to track changes in the aquatic plant community (Rasmussen and Anderson 2005), an attempt was made to support these microfossil-based results with macrofossil data. However, sorting of macrofossils for the purpose of radiocarbon dating revealed that such remains were extremely rare in the sediments of Lake Uddelermeer (Engels et al. 2016).

The sterol and stanol compounds all have low concentrations in this part of the record (Fig. 4), indicating that input of faecal matter from local wildlife into the lake was low. Finally, the algae encountered in the microfossil samples show the presence of green algae *Botryococcus* and *Pediastrum* in relatively low abundances (Fig. 5). These combined results suggest that Lake Uddelermeer might be characterized as a lake with a mix of aquatic macrophytes and abundant phytoplankton during zone 1a, and with an initial increase and subsequent decrease in nutrient availability through time.

Zone 1b: 6000-1030 cal yr BP

Climate model simulations indicate that temperatures in western Europe decreased by $\sim 1^{\circ}\text{C}$ at the end of the Holocene Thermal Maximum at around 6000 cal yr BP (Renssen et al. 2009). A gradual transition is visible in the pollen diagram at 6000 cal yr BP where the main diagram shows a decrease in arboreal pollen (Fig. 5), while a stepwise increase can be seen in the abundance of Ericaceae pollen. This change is accompanied by the first occurrences of crop plants (e.g. Cerealia) in the record, indicating human forest clearance and the establishment of agricultural practices in the area near the lake. The reconstructed onset of forest clearance based on the palynological data corresponds to the age of the oldest archeological finds in the direct vicinity of Lake Uddelermeer, which also date back to ca. 6000 cal yr BP (Groenewoudt et al. 2006). Additionally, the initial increase of epicoprostanol (Fig. 4), a human faecal biomarker (Vane et al. 2010), at 2140 cal yr BP coincides with the appearance of Secale in the pollen diagram, indicating human presence in the area around the lake.

The most prominent aquatic macrophytes in the pollen and spore record between 6000 and 1030 cal yr BP are *Isoetes* and *Littorella* (here together referred to as 'isoetids'; Farmer and Spence 1986; Boston and Adams 1987), while *Nymphaea* and *Potamogeton* disappear. *Isoetes* and *Littorella* are typical inhabitants of oligotrophic lakes that are adapted to nutrient limited conditions. This ability is due to symbiosis with mycorrhiza, their growth form and their slow growth rate (Sand-

Jensen, 1978; Boston and Adams, 1987). Isoetids are common in shallow clear-water environments, although they are tolerant to low-light conditions (Farmer and Spence 1986). *Littorella uniflora* (L.) Asch. grows in shallower habitats (0-2 m) than *Isoetes* (2.0-4.5 m; Sand-Jensen, 1978) and is adapted to tolerate disturbances such as wave action and some sediment redeposition (Farmer and Spence 1986). The loss-on-ignition record from Lake Uddelermeer shows increased variability in organic matter in the lake sediments during 6000-1030 cal yr BP (Engels et al. 2016), which might be the result of the farming activities (triggering erosion) in the area around the lake. The lake's shore area probably had relatively high disturbance rates, and isoetids are considered to be stress-tolerant and capable of growing in such habitats (Rørslett and Brettum 1989). The increased disturbance rate, combined with the low nutrient availability, might have given isoetids a competitive advantage over other aquatic macrophytes between 6000-1030 cal yr BP.

The sedimentary pigment records show decreasing abundances for all pigments during zone 1b with the exception of chlorophyll *b* (Fig. 3). A slight increase in the abundance of *Botryococcus* can be observed from 6500 cal yr BP onward, although this shift is not outside the internal variability of the record (Fig. 5). All these changes indicate a transition from a mesotrophic lake with a mix of aquatic macrophytes and abundant phytoplankton to a lake ecosystem that is characterized by clear-water conditions and a dominance of aquatic macrophytes.

A lake level reconstruction based on a combination of ground-penetrating radar imagery and a comparison of pollen records derived from multiple core sequences indicates a relatively large decrease of lake levels by ~2 m around 3150 cal yr BP (Fig. 6; Engels et al. 2016). This decrease in lake levels was short-lived, lasting for only several hundred years, and could have been the result of decreased levels of precipitation, or of local factors such as an erosional outflow event (Engels et al. 2016). The phase of lake level lowstand was followed by an increase in lake levels at 2850 cal yr BP by ~4 m to levels that are 1.5 m higher than the present day lake level. Engels et al. (2016) attribute the increase in lake levels to increased precipitation amounts, which might have been the result of changes in atmospheric circulation following the solar activity decline at 2850 cal yr BP (van Geel et al. 1996; Engels and van Geel 2012). These relatively large-scale fluctuations in lake levels are to some extent reflected in the high proportion of variance explained by water depth (Table I), although the effects of changes in lake level are not directly apparent from the individual palaeoecological datasets that represent the development of the lake ecosystem. There are two records that do show a response to the lake level lowering at 3150 cal yr BP: first, there is a maximum in the abundance of *Botryococcus* between 3150-2800 cal yr BP; second, the chironomid records show changes in the abundance of several key-taxa, with an increase in shallow-water taxa such as *Lauterborniella* and *Parakiefferiella bathophila*-type (Fig. 5).

Zone 2: 1030 cal yr BP-present

The strongest changes in our data can be seen between 1120 and 940 cal yr BP (mean: 1030 cal yr BP (920 AD)), where our combined results likely reflect the transition from a clear-water shallow lake ecosystem that was relatively rich in aquatic macrophytes to a turbid phytoplankton-dominated state. We observe a sharp decrease in arboreal pollen (Fig. 5) and a short-lived minimum in carbon content of the sediment (Fig. 6). The aquatic dataset shows a clear change in dominant taxa as well, with decreases of *Isoetes* and *Littorella* between 1120 and 940 cal yr BP. *Potamogeton*, already present in the lake since 3100 cal yr BP, becomes the most abundant aquatic macrophyte in the pollen record (Fig. 5). Mass blooming of phytoplankton, most likely as the result of eutrophication of the water body, might have outshaded submerged macrophytes such as *Isoetes*, which decrease from 1030 cal yr BP onward (Fig. 5). *Pediastrum*, *Scenedesmus* and *Tetraedron minimum* all show abrupt increases at this time, and sedimentary pigments that are indicators of cyanobacteria (zeaxanthin), diatoms (diatoxanthin) and more generally of chlorophytes all show abrupt and strong increases in concentrations at this point as well (Fig. 3).

Additionally, the chironomid record shows strong changes across the 1120-940 cal yr BP interval. Almost all taxa that had high percent-abundances in the period before this interval decrease or disappear. This includes taxa that are associated with aquatic macrophytes such as *Endochironomus albipennis*-type and *Parakiefferiella bathophila*-type (Brooks et al. 2007; Moller Pillot 2009; 2013). The taxa that increase in abundance include *Ablabesmyia* and *Procladius*. Although *Ablabesmyia* is often associated with macrophytes as well, both these taxa are typically interpreted to be generalists that can thrive in habitats where many other taxa are unable to successfully complete their life cycles (Moller Pillot and Vallenduuk 2007). *Microtendipes pedellus*-type is another taxon that increases in abundance after ca. 1000 cal yr BP (Fig. 5), and this taxon is often encountered in dynamic environments (Engels et al. 2008a; 2008b), and it is often associated with clastic sediments (Brooks et al. 2007). The upper part of the sediment record of Lake Uddelermeer still consists of organic-rich gyttja, and the increased abundances of *M. pedellus*-type is slightly unexpected given its preference for mesotrophic conditions and a stable oxygen regime (Moller Pillot 2009). The increase in *M. pedellus*-type could be a relative increase rather than an absolute increase in numbers, due to the disappearance of taxa that are less tolerant of disturbances.

As an alternative explanation for the decrease in abundance of the isoetids, eutrophication might have led to the isoetids being outcompeted by faster-growing macrophytes or unable to

1 outgrow the epiphytes on their leaves, as has been shown for Scottish lakes (Farmer and Spence,
2 1986). However, in the case of Uddelermeer we do not find evidence for the colonization of any new
3 aquatic macrophyte around 1030 cal yr BP. The increase in *Myriophyllum alterniflorum* only occurs
4 at 700 cal yr BP, several centuries after the observed decrease in the isoetids. A similar (but later)
5 shift in the aquatic flora of Dutch lakes was observed by Roelofs et al. (1984), who showed that 20th
6 century phosphate enrichment of lake sediment in a number of Dutch lakes lead to luxuriant growth
7 of rooted macrophytes such as *M. alterniflorum*, replacing macrophyte stands belonging to the
8 phytosociological alliance *Littorellion*.
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10 The identification of a major eutrophication-related lake ecosystem during the Medieval
11 period is unexpected given the abundant evidence of 18th-20th century changes in The Netherlands
12 (Kirilova et al. 2010a; 2010b) and beyond (Lotter 2001; Millet et al. 2010). However, Bradshaw et al.
13 (2005a; 2005b) reported a similar result where they show major eutrophication and ecosystem
14 changes during the Medieval period for a shallow Danish site, and several studies on other sites in
15 northwest Europe have since reported similar results (Bjerring et al. 2008; Hübener et al. 2009;
16 Meyer-Jacob et al. 2015). Additionally, Thienemann et al. (2017) reconstructed the impact of
17 anthropogenic activity on the environment of Lake Dorjan on the Macedonian/Greek border using a
18 combination of proxy-indicators that includes palaeoecological indicators (e.g. pollen) as well as
19 novel geochemical techniques (e.g. faecal steroids), similar to this study. Thienemann et al. (2017)
20 recognized phases of increased faecal steroid concentrations from the early Bronze Age onward,
21 where we also find an early increase in epicoprostanol concentrations at 2140 cal yr BP (Iron Age).
22 Thienemann et al. (2017) also reconstruct increased human activity during the Medieval period and
23 the Modern era, similar to the results reported here as well as those by Bradshaw et al. (2005b).
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41 Identification of natural and anthropogenic drivers of ecosystem change

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44 A CCA with PCA-scores of the sterol and stanol concentrations as the single explanatory variable
45 shows that faecal input is not the sole driver of the ecosystem shift at 1030 cal yr BP (Table I), which
46 contrasts locally held opinions of 20th century pollution of the lake. This was also apparent from the
47 faecal biomarker data, which show peak abundances of e.g. coprostanol (suggesting contamination
48 by omnivore faeces, i.e. human or pig) after -50 cal yr BP, whereas the lake already underwent a
49 major ecosystem shift much earlier on. Similarly, the retting of hemp in Lake Uddelermeer also
50 started to take place after the ecosystem had already changed, and the CCA run with *Cannabis*-type
51 pollen as the sole explanatory variable indeed confirms that retting was not the driver of the
52 ecosystem phase-shift (Table I). In fact, the results of our numerical analyses suggest that changes in
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the 'aquatic dataset' can only be explained by environmental factors that show gradual long-term development: sediment age and water depth. The results suggest that when the long-term development is partialled out (by including age or water depth as a co-variable in the CCAs) there is no single factor that caused the ecosystem shift around 1030 cal yr BP, both when analyzing the full Holocene dataset as well as when analyzing a truncated dataset spanning 1710 cal yr BP to the present. Only a combination of environmental factors can explain the observed changes, but with a p-level that is higher than those of the runs with age or water depth as sole explanatory variables. It is important to remember that the set of 'drivers' used in our tests may not have captured all the factors that might have influenced the lake ecosystem, as a variety of different anthropogenic and natural causes might have impacted the lake on different temporal and spatial scales.

Implications of the reconstructed eutrophication history of Lake Uddelermeer

The implications of our study are two-fold: first, our results show that for lakes such as Uddelermeer a commonly used date such as 1850 AD (Battarbee 1999; Bennion et al. 2004) as representative of a 'predisturbance state' or of 'baseline conditions' of the lake ecosystem is incorrect. This result is in line with those from other parts of Europe (van Geel et al. 1994; Bradshaw et al. 2005b; Bjerring et al. 2008; Hübener et al. 2009; Hillbrand et al. 2014; Meyer-Jacob et al. 2015; Thienemann et al. 2017), where lake ecosystems also underwent major changes as a result of changing agricultural activities (including the retting of hemp) from the mid-Holocene onward, significantly influencing trophic levels of the studied lakes prior to 1850 AD. In the case of Lake Uddelermeer, it is actually difficult to determine what the 'natural' state of the lake ecosystem is, as even the occurrence of isoetids from 6000 cal yr BP onward seems to be linked to the occurrence of humans and the erosion of sandy soils in the area around Lake Uddelermeer. While several other palaeolimnological studies of Holocene shifts in trophic status of a lake due to human disturbance have also indicated early (pre-Medieval) changes in lakes in e.g. Switzerland (Dapples et al. 2002; Lotter and Birks 2003), the example of Lake Uddelermeer presented here to our knowledge represents one of the earliest anthropogenically driven shifts in lake status in western Europe.

Second, our results indicate that the ecosystem phase-shift at 1030 cal yr BP already occurred under (probably) relatively weak forcing when compared to modern-day nutrient input into the lake ecosystem. This means that restoration measures such as proposed in the last decade of the previous century (i.e. removal of the top part of the sediment body; Grontmij 1996; Bohncke 1999) would most likely have been insufficient to force the lake back into an oligotrophic clear-water state with abundant isoetids.

Conclusions

We reconstructed the long-term (Holocene) ecosystem development of Lake Uddelermeer using palaeoecological and novel geochemical techniques, and show that the ecosystem of Lake Uddelermeer was characterized by a mix of aquatic macrophytes and abundant phytoplankton between 11,700 and 6000 cal yr BP. Increasing human impact on the landscape around Lake Uddelermeer is observed between 6000 and 1030 cal yr BP (decrease in arboreal pollen, first occurrences of crop plants), and the lake ecosystem changed to clear-water conditions and a dominance of submerged aquatic macrophytes including isoetids. The lake's shore area probably had high disturbance rates, which, combined with the low nutrient availability, might have given isoetids a competitive advantage over other aquatic macrophytes. The strongest changes in our data are observed around 1030 cal yr BP (~920 AD) when most algal microfossils, the sedimentary pigments, the plant and faecal biomarkers, and the chironomid record all show abrupt and dramatic changes, likely reflecting the transition from a clear-water shallow lake ecosystem that was rich in aquatic macrophytes to a turbid phytoplankton-dominated state.

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Table I

Results of direct gradient analyses (canonical correspondence analyses (CCAs)) used to determine the effects of the individual environmental variables on the 'aquatic dataset', which includes the percent-abundance data of the pollen of aquatic macrophytes, algal microfossils (both expressed against the pollen sum) as well as the concentrations of the sedimentary pigments. Two sets of analyses were performed, one with the complete 'aquatic dataset' ('full') which covers the entire Holocene period, and one set with the youngest 20 samples only ('n=20'), spanning the interval between 1710 cal yr BP and the present. Environmental (predictor) variables: age (AGE; long term trends); percent-abundance of tree pollen (%AP; regional vegetation); total organic carbon content (CARB, catchment-related processes); PCA-scores of the sterol and stanol concentration data (FAEC, influx of plant and faecal matter); percent-abundance of *Cannabis*-pollen (%CAN, retting of hemp) and water depth (H2Odepth; habitat development). The table presents the proportion of the variance of the 'aquatic dataset' explained by different sets of environmental factors and the significance level of the relationships assessed using anova (n=999). P levels and significance follow the output as produced by the vegan library in R (Oksanen et al. 2016), where '***' = 0 - 0.001; '**' = 0.001 - 0.01; '*' = 0.01 - 0.05. P levels >0.05 are considered as not significant. %AP and %CAN are not used in combination, as these parameters are not independent; similarly, AGE and H2Odepth co-vary and have not been used together. A set of CCA runs as listed under (2) where age was replaced with H2Odepth showed similar results and is not shown in the table.

Run	Predictor(s)	Co-variable(s)	'Aquatic dataset'-full %-explained	p-level	'Aquatic dataset'-n=20 %-explained	p-level
(1)	AGE	None	21.6	0.001***	24.0	0.001***
	%AP	None	7.3	0.149	6.9	0.299
	CARB	None	4.7	0.341	7.9	0.185
	FAEC	None	7.8	0.094	7.1	0.280
	%CAN	None	7.1	0.166	8.9	0.161
	H2Odepth	None	25.3	0.001***	19.0	0.004**
(2)	%AP	Age	4.3	0.325	8.7	0.145
	CARB	Age	4.8	0.248	7.4	0.189
	FAEC	Age	3.1	0.442	8.3	0.138
	%CAN	Age	6.4	0.156	8.3	0.159
(3)	%AP, CARB	Age	8.5	0.333	14.6	0.167
	%AP, CARB, FAEC	Age	18.5	0.049*	27.2	0.007**

Figure captions

Fig. 1: Location of Lake Uddelermeer (The Netherlands).

Fig. 2: Bayesian age-depth model for the central core sequence from Lake Uddelermeer (Engels et al. 2016), generated using OxCAL v. 4.2 (Bronk Ramsey 2009)

Fig. 3: Concentrations of sedimentary pigments per gram OC extracted from the Holocene sediments of Lake Uddelermeer plotted on an age-scale, with a sediment depth scale added for reference. Note the different scaling used for the x-axes.

Fig. 4: (A) Concentrations of plant and faecal biomarkers per gram OC extracted from the Holocene sediments of Lake Uddelermeer plotted on an age-scale, with a sediment depth scale added for reference. Note the different scaling used for the x-axes. (B) Same as (A), but with a focus on the last 2 millennia, showing details of changes that occurred in the past ~100 years.

Fig. 5: Summarizing palaeoecological diagram for the Holocene record of Lake Uddelermeer on an age-scale (cal yr BP) showing selected crop plants, aquatic macrophytes and algal taxa (microfossils) and chironomids. A sediment depth scale (cm) is shown for comparison. All microfossils curves are shown as percentages of the pollen sum (which includes the pollen of trees and shrubs, Ericaceae and upland herbs) with a 5x exaggeration. Microfossils are grouped according to ecological preferences of the taxa. Note the different scaling used for the x-axes of the algal taxa. The chironomids are plotted as a percentage-abundance of the total number of chironomid head capsules per sample. Scores of samples on the first axis of a Principal Component Analysis (PCA) that uses all chironomid taxa is shown to the right of the figure.

Fig 6: Summary diagram of the pollution history of Lake Uddelermeer. (A) The six explanatory variables used in the variance portioning tests: age (cal yr BP), arboreal pollen (%), organic carbon content (%), *Cannabis*-type pollen (%), the scores of the sterol and stanol concentrations on the 1st PCA axis and water depth (m). (B) Selected curves reflecting the status of the lake ecosystem: *Isoetes* spores (%), *Scenedesmus* (%) and scores of the sedimentary pigments on the 1st PCA axis. (C) Scores of chironomid samples on the 1st PCA axis. Curves for (C) and (D) after Engels et al. (2016). Zonal transitions: zone 1a - zone 1b: 6000 cal yr BP; zone 1 - zone 2: 1030 cal yr BP. Geological and archaeological zonation is plotted for reference.

Figure 1

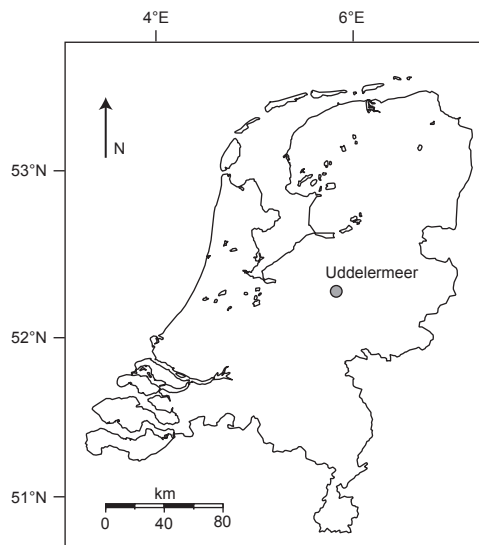


Figure 2

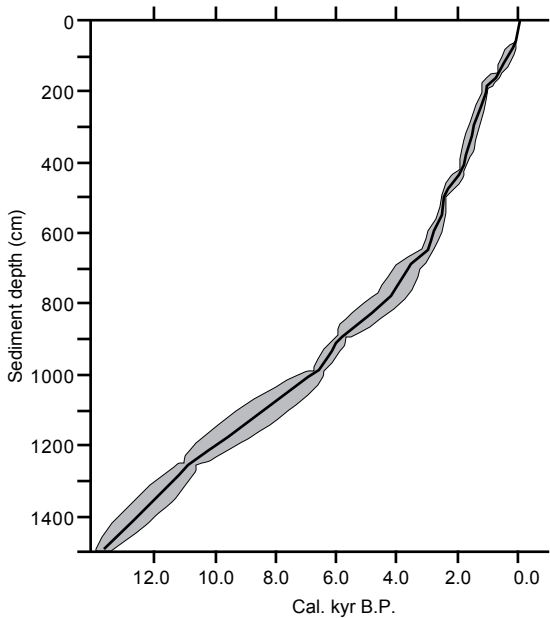


Figure 3

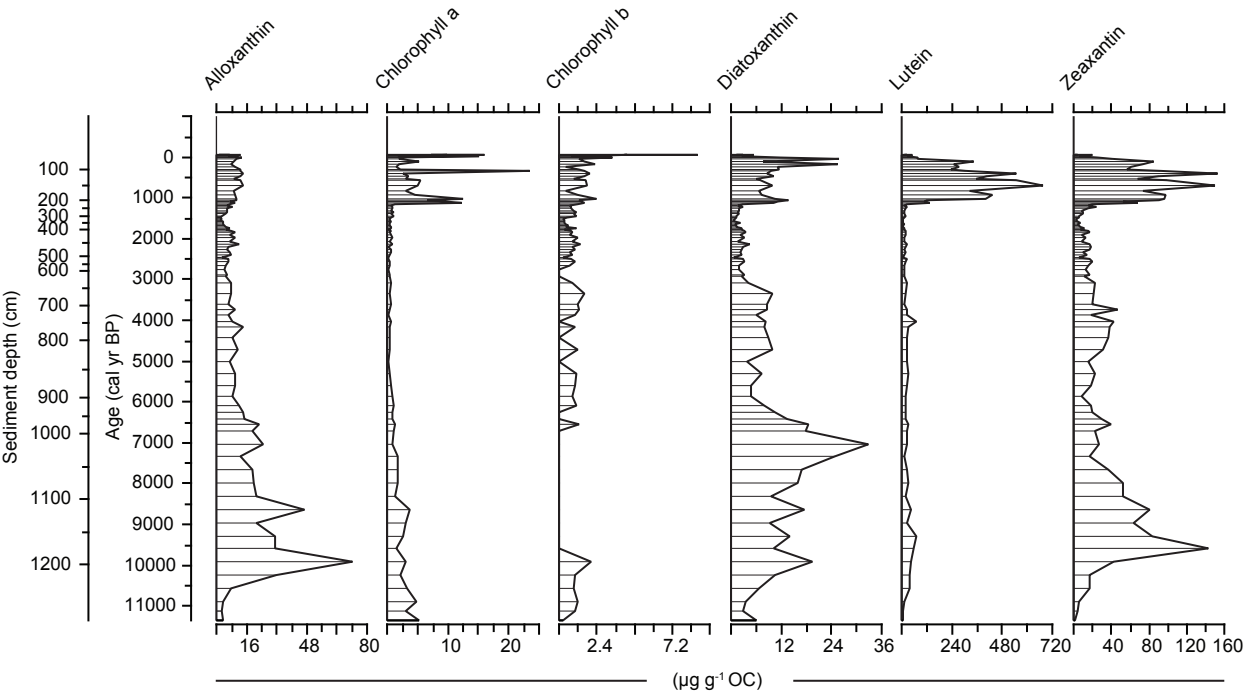


Figure 4

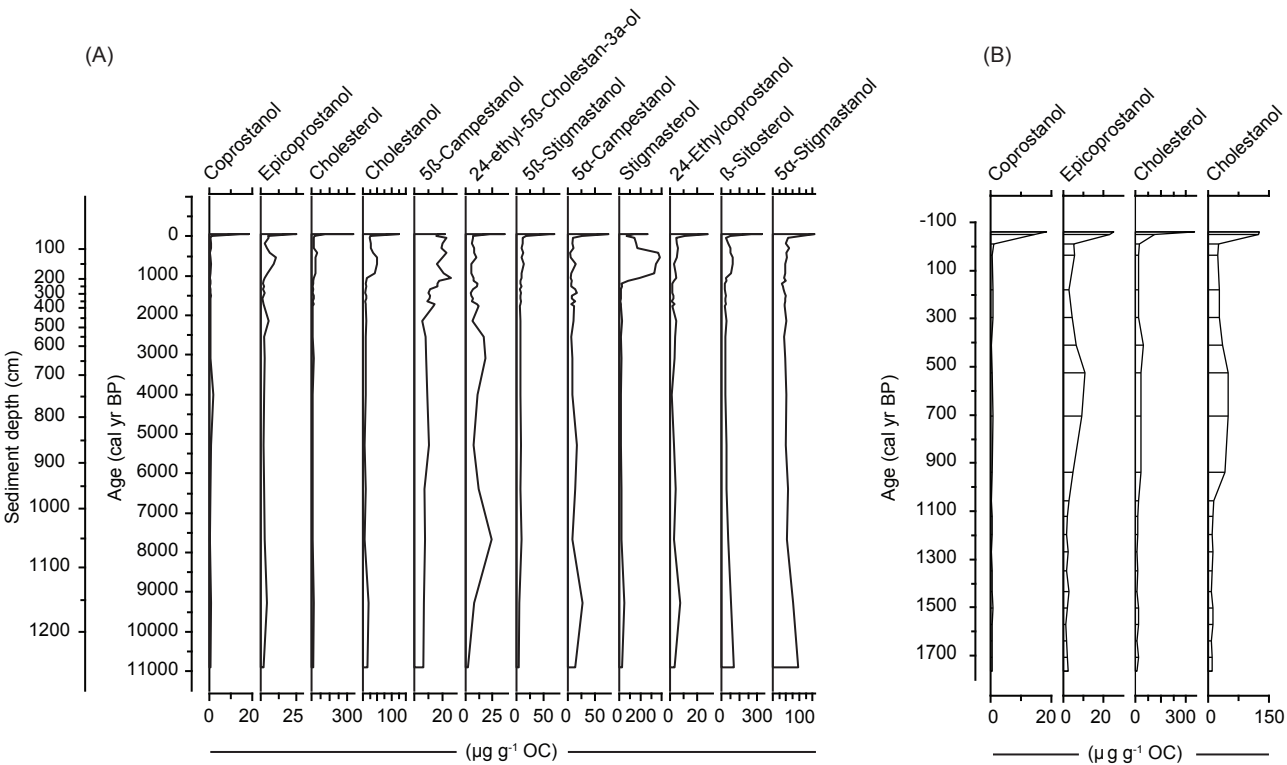
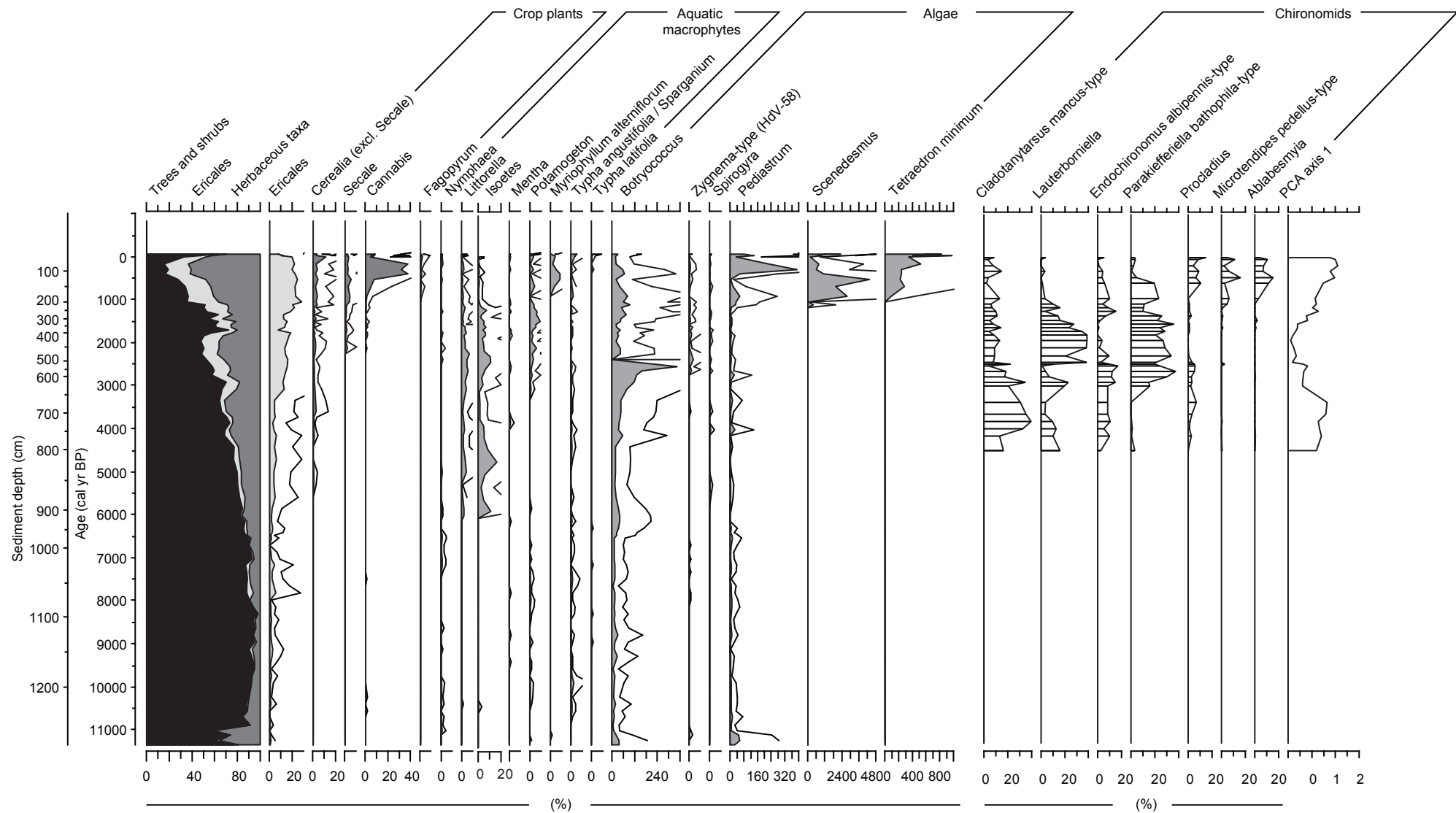


Figure 5



(C) Chironomidae

